view of Alexander *et al.* (*J. of Bacteriology, 176,* 7079 (1994)). In addition, the Examiner rejected claims 11, 22 and 29 under 35 U.S.C. § 103(a) as being unpatentable over McLaughlin *et al.* and Alexander *et al.* in view of Świerzko *et al.* (*Infection and Immunity, 61,* 3216-3221 (1993)). Claims 6-8, 11-12, 18-29 have been cancelled, thus rendering these rejections moot. Insofar as these rejections may be maintained with respect to the pending claims, they are respectfully traversed.

Claim 30 is directed to a process for the production of a *H. influenzae*-specific LOS that involves growing gram-negative bacteria containing (i) a core lipid structure containing a terminal heptose, (ii) a DNA sequence having a *rfe* gene, and (iii) an isolated DNA sequence with a lipooligosaccharide-synthesis gene G (*lsgG*) from *H. influenzae.* The *lsgG* encodes LsgG, which regulates *rfe*, such *H. influenzae*-specific LOS is synthesized by the addition of an acceptor molecule to the terminal heptose molecule, and recovering the *H. influenzae*-specific LOS from the culture medium.

Claim 38 is directed to a process for the production of a complex carbohydrate involving growing in a culture medium gram-negative bacteria with a core lipid structure containing a terminal heptose and (i) a DNA sequence having a *rfe* gene and (ii) an isolated DNA sequence having a *lsgG* from *H. influenzae*, wherein *lsgG* encodes LsgG, and wherein *rfe* is regulated by LsgG such that complex carbohydrate is synthesized by the addition of an acceptor molecule to the heptose molecule and recovering the complex carbohydrate from the culture medium.

Claim 47 is directed to a method of modifying a terminal heptose of a lipopolysaccharide (LPS) or lipooligosaccharide (LOS) core structure of a gram-negative bacterial species containing a *rfe* gene. The method involves regulating the *rfe* gene with a protein encoded by an isolated *lsgG* gene from *H. influenzae* such that N-acetyl glucosamine is added onto the terminal heptose.

Applicants' claims are directed to the enzymatic synthesis of complex carbohydrates. These claimed processes involve the use of a *rfe*, a gene encoding UDP-GlcNac:Undecaprenol GlcNac-1 phosphate transferase, and *lsgG*, which encodes LsgG (see for example the specification at page 4, line 29-page 5, lines 3, and Attachments 1-2 enclosed herein). Applicants disclose that UDP-GlcNac:Undecaprenol GlcNac-1 phosphate transferase catalyzes

the transfer of N-acetyl glucosamine onto the carrier lipid undecaprenol phosphate (page 7, lines 9-11 and Attachment 2). Applicants further disclose that expression of *rfe* is controlled by LsgG, a regulatory protein (page 7, lines 11-12 and Attachment 1). By exploiting this interaction between *rfe* and *lsgG*, Applicants disclose that complex carbohydrates, such as a *H. influenzae*-specific LOS, can be synthesized onto a core lipid structure containing a terminal heptose.

2. McLaughlin *et al.* in view of Alexander *et al.*

McLaughlin *et al.*, of which Dr. Apicella (one of the co-inventors of the present application) is a co-author, disclose the DNA sequence of the *H. influenzae* LOS synthesis genes (*lsg*) locus (GenBank accession number M94855) (page 167 of McLaughlin *et al.*). McLaughlin *et al.* further disclose a restriction map of the *lsg* locus showing seven complete open reading frames (ORF) and one partial ORF (abstract; Figure 1). In addition, McLaughlin *et al.* disclose that *H. influenzae* type B (Hib) containing mutant *lsg* loci were prepared by shuttle mutagenesis and the mini-Tn3(Cm) transposon (abstract; pages 166-167; Figure 7A and B). ELISA analysis of the LOS produced by the mutant Hib strains (2101, 2108 and 2110) revealed altered binding to the monoclonal antibodies 6E4 and 2F2 as compared to an isogenic Hib strain (A2).

There is nothing, however, in McLaughlin *et al.* that teaches or suggests a process for the production of *H. influenzae*-specific LOS that employs a *lsgG* gene; a method of modifying a terminal heptose; or a process for the production of a complex carbohydrate. Moreover, there is nothing in McLaughlin *et al.* which discloses *rfe*, let alone the interaction between *rfe* and LsgG. At page 3 of the Office Action, the Examiner asserts that McLaughlin *et al.* teach that "sugar transferases expressed from the *lsg* are responsible for the modification of the existing *E. coli* LPS." Applicants respectfully submit that regarding characterization of the *lsg* locus, McLaughlin *et al.* were not at all sure of what the ORFs in the locus encoded. McLaughlin *et al.* states that

> [t]he sequence analysis of the 7.4 kb fragment and database search for the proteins homologous with the 8 ORFs, however, failed to show significant high homology to any known sugar transferase, and it was not possible to deduce the functions of the products of the ORFs based on the sequence homology" (emphasis added, page 172).

McLaughlin *et al.* further disclose that "future studies will be directed at defining the functions of the proteins expressed by the ORFs within this locus" (page 174). Thus, the pending claims are not obvious over McLaughlin *et al.*

Alexander *et al.* do not remedy the deficiencies of McLaughlin *et al.* To illustrate Alexander *et al.*, the Examiner's attention is respectfully directed to Attachment 3, enclosed herein. Alexander *et al.* disclose that *rfe* is involved in the biosynthesis of the enterobacterial common antigen (ECA), of the O7 repeat of *E. coli* as well as other O types, *e.g.*, O18, O75 and O111 (page 7079, right hand column). However, Alexander *et al.* do not teach or suggest the use of LsgG, let alone the interaction between *rfe* and LsgG. Therefore, Alexander *et al.* do not obviate the claimed invention.

Therefore, withdrawal of the 35 U.S.C. § 103(a) rejection of the pending claims over McLaughlin *et al.* in view of Alexander *et al.* is respectfully requested.

3. McLaughlin *et al.* and Alexander *et al.* in view of Świerzko *et al.*

McLaughlin *et al.* and Alexander *et al.* are discussed above.

Świerzko *et al.* do not remedy the deficiencies of McLaughlin *et al.* or Alexander *et al.* Świerzko *et al.* disclose the serological characterization of antisera collected from rabbits immunized with heat-killed *Salmonella minnesota* R4 chemotype Rd_2P^- (page 3218). In a passive hemolysis assay, Świerzko *et al.* disclose that the smallest structure recognized by the antisera was Hep-Kdo-GlcNhm$_2$, and the smallest structure reacting in an EIA was Hep-Kdo disaccharide. However, there is nothing in Świerzko *et al.* that teaches or suggest a process for the production of a LOS, let alone *H. influenzae*-specific LOS. Moreover, Świerzko *et al.* do not teach or suggest such a process that uses *rfe* or *lsg*. Therefore, for these and the reasons above, the pending claims are not obvious over the combination of McLaughlin *et al*, Alexander *et al.* and Świerzko *et al.*

Withdrawal of the 35 U.S C. § 103(a) rejection is respectfully requested.

Conclusion

Applicants respectfully submit that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicants' attorney (612-373-6961) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.,

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
612-373-6961

Date 5 May 2003 By Ann S. Viksnins
Ann S. Viksnins
Reg. No. 37,748

The undersigned hereby certifies that this correspondence is transmitted by facsimile (FAX NO. 7903-872-9306) to: Attn.: Examiner Yong Pak, GAU 16152, Commissioner of Patents, P. O. Box 1450, Alexandria, VA 22313-1450, on this 5th day of May, 2003.

Candis B. Buending Candis Buending

Name Signature